Protocol: Desalting of 40 nmole Scale Oligonucleotides

Materials:

- Sep-Pak Plus C18 cartridges (1 per oligo), Waters catalog #WAT020515 (box of 50).
  methanol
- 60% v/v methanol in H20
- 5M NaCl
- Sterile distilled H20

Procedure:

1. Resuspend the dried oligo using 1 ml H20, add 110µl 5M NaCl. Yortex to dissolve, transfer to a microfuge tube and centrifuge 2-3 min to remove insoluble particles.

2. Attach a syringe to the Sep-Pak, wash cartridge with 5 ml Methanol, then with 5 ml sterile distilled H20. Push all the fluid through the cartridge.

3. Load the resuspended oligo onto the cartridge, slowly push all fluid through.

4. Wash cartridge with 3 ml sterile distilled H20.

5. Place a clean tube under the cartridge and elute the oligo with 1.5 ml 60% methanol. Push all of the fluid out of the cartridge.

6. Dry the eluted oligo in a speed-vac. Resuspend using 0.5 ml of H20 or buffer. Dilute 10 µl into 1 ml of H20 and measure the absorbance at 260 nm. Calculate the concentration as shown below.

Quantitation:

Quick calculation, close enough for PCR:
Concentration in µg/ml = (A260 of 10 µl in 1 ml) x 3,300

More accurate method:
Concentration in pmole/µl = (A260 of 10 µl in 1 ml) x 10,000
divided by:
[1.54 nA + 0.75 nC + 1.17 nG + 0.92 nT]
where n equals the number of occurrences of a nucleotide in the sequence

Notes:
0.2 µmole oligos may also be desalted using this protocol, however the cartridge may not bind all of the DNA. The material that flows through the cartridge during the load/wash step should be saved, quantitated and, if neccessary, applied to a second Sep-Pak. For 1 µmole or larger scales, Waters manufactures larger Sep-Pak columns. See us for information.

http://metalab.unc.edu/nacf/

NEW! Check out our new online order form! NEW!

Link to Oligo Calculator

ATTENTION:The Nucleic Acid Core Facility is pleased to announce that we have purchased a Perkin Elmer Applied Biosystems Model 3948 Synthesizer. This machine enables synthesis and purification of 50mers and shorter at a 40 nmole scale with no extra charge to the researcher. We encourage UNC researchers to take advantage of this new service beginning March 1st, 1999.

PRICE REDUCTION!

Beginning May 1st, the Nucleic Acid Core Facility will be able to offer 40 nM scale oligos at at reduced price for Cancer Center members. Our new price will be 80 cents per base. Purification will remain free of charge for those 40 nM scale oligos less than 50 base pairs. This price change is not a promotion. It is our new, regular price.

Lineberger Nucleic Acids Core Facility

General Information

Mission:

The Lineberger NACF provides custom synthetic oligonucleotides to researchers at the University of North Carolina at Chapel Hill. We operate three Perkin Elmer Applied Biosystems model 394 DNA synthesizers and one ABI model 3948.

Location:

Rooms 228 and 232 of the Lineberger Comprehensive Cancer Center

People:

Dr. Qing Yang
Mitch Sally
Michael Townsend

Hours of Operation:

8:30 a.m. to 5:00 p.m.
Monday through Friday

Telephone:

(919) 966-5624

Fax:

(919) 966-0060

Prices (per base):

Scale	Center Members	Non-Members
40 nmole	$0.80	$1.15
0.2 µmole	$1.45	$1.75
1.0 µmole	$3.00	$3.60
2.5 µmole	$3.50	$4.20
10 µmole	$12.00 + $32/oligo	$15.00 + $32/oligo

Click here to see a list of modifications and their prices.

Ordering information:

Oligos may now be ordered via the internet.

Please follow this link to place your order now.

Oligo orders can still be dropped off at the facility or faxed to the number listed above. Turnaround time for 40 nmole orders averages 2 days. We will call you when your oligo is ready. If you prefer, we will respond by email.

Please use a separate order form for each oligonucleotide. If you need a form follow the link below and print a copy.

Click here to see the form

Please fill out the form carefully according to the following instructions:

Name: Requestor's name.
Building/Room#: Location of the requestor's laboratory. If this is your first order, please provide us with your department name and campus box address.
Billing PI (Full Name): Name of the principle investigator to whom the bill should be sent.
Phone #: We will call this number when the oligo is ready.
Date: This is for your use.
Date Received: This is for our use, and is stamped on the form when the order reaches us.

Length: ___mer Number of bases in the requested oligo.
Sequence (In Triplets 5' -->3') Please write clearly using A, g, C, T or the IUB degeneracy codes shown at the bottom of the order form. There is no charge for degeneracies in positions other than the 3' end.
List Modifications: If you have included any non-ATgC nucleotides in your sequence, or if you are requesting end labeling with biotin, fluorescein, phosphate, etc, indicate them here and in your sequence. Click here to see a list of modifications and their prices.
Optional Purification: If you want us to carry out "Trityl On" purification (**free for 40 nmole**, $20 for .2 µmole and $25 for 1.0 µmole & 2.5 µmole) or SepPak desalting ($10 for 40 nmole & .2 µmole and $15 for 1.0 µmole & 2.5 µmole), circle as appropriate. Desalting is unavailable for 40 nM scale oligonucleotides less than 50 base pairs. Trityl-on purification (free of charge to the user) will be substituted for desalting in these cases. For 40 nM scale oligonucleotides greater than 50 base pairs, desalting is performed manually, and the cost to the user is $10. (These options are discussed more completely below)
Designation: Your name for the oligo. Please make this 8 characters or less.
Scale of Synthesis: Scale refers to the theoretical molar yield of the synthesis, although in most cases the actual yield of a synthesis is significantly lower. The approximate molar yields for each of the available synthesis scales are as follows:

Synthesis scale	Approximate Yield
40 nmole	20-40 nmole
0.2µmole	0.15 µmole
1.0 µmole	0.5 µmole
2.5 µmole	1.25 µmole
10 µmole	4 µmole

The 40 nmole scale is appropriate for most sequencing and PCR applications. A 25-mer synthesized at this scale will provide about 250 µg of crude DNA.

Final products:

For 40 nmole purified oligos -- resuspend the oligo in 1ml H_2O or buffer. The concentration of the oligo in pmole/µl is measured for you (check the bottom of your request form).

For 40 nmole crude oligos -- the actual concentration is usually higher than that measured by the synthesizer. Check the concentration by spectrophotometer.

For oligos at 0.2 µmole or higher scale -- check the concentration by spectrophotometer.

Oligo Data Sheets

For 40 nM scale oligos synthesized on the Perkin-Elmer 3948, we no longer provide oligo data sheets. Molecular weight, melting temperature, and GC content can be determined by using the oligo calculator, links to which can be found on this homepage.

Purification Options:

There are several options available for purification of oligonucleotides. The NACF offers two forms of purification. Any oligo can be desalted and oligos which are 50mers or shorter can be purified utilizing trityl-on synthesis and cartridge purification. For oligos shorter than 50mers and at 40nmole scale, synthesis and purification will on done on the synthesizer 3948 (purification will be free). For oligos longer than 50 nucleotides we suggest either PAGE gel purification or Ethanol precipitation. Protocols for each can be accessed by clicking on the words above. The two types of purification that we perform are described below.

Trityl-On Purification:

During oligo synthesis, each coupling step has an efficiency of 98 to 99%. The 1-2% of oligos that do not couple at each step are inactivated and remain in the final synthesis product; these are called n-minus products. In long oligos, the amount of n- material can be

significant. As an example, consider a 75-mer synthesized with 99% efficiency at each step. The amount of full length product will be (.99)75 = 47%. The remaining 53% of the final synthesised product consists of n- products. Because synthesis proceeds from 3' to 5', the n- products consist of deletions from the 5' end. For many applications, this large amount of n- product is not a problem. Occasionally, however, the n- product will interfere and must be removed. Before synthesis starts the NACF can set the machines to add the trityl groups. Purification can then be accomplished by leaving the trityl group on the 5' nucleotide at the end of synthesis. The details of the trityl group would take several paragraphs to explain; suffice it to say that if the "Trityl On" option is selected, only full length synthesis products will contain a trityl group. Since only the full-length oligo will have a trityl group, it can be purified away from n- products through the use of a small chromatography cartridge under conditions that favor trityl binding. Non-trityl containing oligos (the n- products) are not bound and are washed away. The trityl group is then chemically cleaved and remains bound to the cartridge while the full-length oligo is eluted and collected. Contaminating salts are also removed during this process.

Please note that the yield of a purified oligonucleotide will be considerably lower than that of a crude oligo. Typically 1 to 4 OD units will be obtained for purified oligos.

Trityl-On purification protocol.

SepPak Desalting:

In order to Desalt an oligo, the DNA is loaded onto a cartridge in an aqueous solution and binds to the matrix of the cartridge while contaminating salts and very small failure sequences are washed through the cartridge. The oligo is then eluted using a methanol/water soution then dried for storage.

Click here for SepPak desalt protocol.